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MECHANICAL DEVICES TO EXPEDITE BOLL WEEVIL REARING IN THE LABORATORY

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Cultures of the boll weevil (Anthonomus grandis Boheman) have been maintained since 1958 at the Southwestern Cotton Insects Investigations Laboratory at College Station, Tex., according to methods developed by Vanderzant and Davich.[2/] More recently, most of the other State and Federal laboratories where experimental work on this insect is being conducted have also established such weevil cultures. Because weevil eggs are deposited one at a time and the larvae are cannibalistic, rearing methods have been developed for handling the insects as individuals rather than as a mass. Such methods are expensive to carry out because they require much hand labor.

Several simple mechanical devices that materially reduce the cost of rearing the boll weevil have been developed at the Boll Weevil Research Laboratory, State College, Miss. These devices, along with the procedures for utilizing them, are described in this report.

EQUIPMENT FOR PREPARING DIET MEDIUM

Although a chemically defined larval diet is available for rearing the boll weevil[3/] preparation of this diet is complicated and expensive. Earle et al.[4/] showed that ground cotton squares extracted with acetone were a good source of protein in the larval diet. Small quantities of this powder can easily be prepared in the laboratory

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1/ In cooperation with the Mississippi Agricultural Experiment Station. The authors are at the Boll Weevil Research Laboratory, State College, Miss.

2/ Vanderzant, Erma S., and Davich, T.B. Laboratory rearing of the boll weevil: A satisfactory larval diet and oviposition studies. Jour. Econ. Ent. 51: 288-291. 1958.

3/ Vanderzant, Erma S., unpublished report.

4/ Earle, N. W., Gaines, R.C., and Roussel, J. S. A larval diet for boll weevils containing an acetone powder of cotton squares. Jour. Econ. Ent. 52: 710-712. 1959.

by using a Waring Blendor[5] and a Buchner funnel. However, several hundred pounds of square powder were needed for the mass-rearing program at State College, Miss. For this purpose, a more economical means of producing the powder was devised.

Apparatus for Preparing Square Powder

Three-quarters of a ton of cotton squares was collected and immediately frozen, since the process of freezing and subsequent thawing disrupts many of the cells and thus aids in the extraction. Cotton squares to be processed were removed from the freezer and covered with acetone. Much of the free water in and on the squares mixed with the acetone and was then drained from the squares along with the acetone. The partially dehydrated squares, covered with twice their volume of fresh acetone, were then ground for 5 minutes in gallon-sized blenders. After the grinding was completed, the slurry was poured into a large muslin sack and most of the acetone allowed to drain off into a vat. If the initial dehydration step described above was omitted, the water present in the slurry caused swelling of the muslin fibers of the sack so that rapid draining was not possible.

After the contents of the muslin sack had drained, the residue was transferred to a large screw press (fig. 1), where the remainder of the acetone was squeezed from the plant material. The process of grinding, draining, and squeezing was repeated and the resultant powder was allowed to air-dry to remove the last traces of acetone. When the acetone was removed from small quantities of square powder by means of a Buchner funnel and vacuum, three extractions were usually required; but when the screw press was used, only two extractions were necessary to produce an equivalent product.

Apparatus for Preparing Germinated-Cottonseed Puree

Vanderzant and Davich[6] developed a medium that could be used to replace the bolls or squares as adult weevil food and as oviposition sites for the females. One of the necessary ingredients was a puree made from dehulled, germinated cottonseeds. Different methods of separating the hulls from the meats were tried, but the only satisfactory method required that the seeds be squeezed one at a time by hand. Because large quantities of germinated seeds were necessary for mass rearing, a mechanical means of dehulling the seeds was developed.

Two stainless-steel rollers, each measuring 6 inches in diameter and 6 inches in length, were mounted in a stainless-steel frame so that a 1-mm. gap remained between the rollers. The roller shafts were connected by gears so that they rotated in opposite directions when turned by a handle (fig. 2).

5/ Mention of proprietary products in this report does not constitute their endorsement by the U. S. Department of Agriculture.

6/ Vanderzant, Erma S., and Davich, T. B. Artificial diets for the adult boll weevil and techniques for obtaining eggs. Jour. Econ. Ent. 54: 923-928. 1961.

Figure 1.--Large screw press used to squeeze acetone from ground cotton squares.

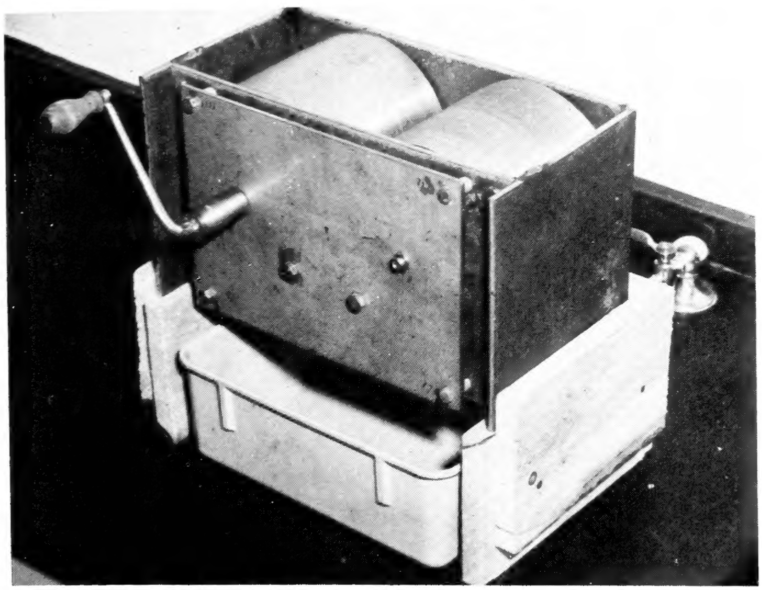

Figure 2.--Stainless-steel rollers used to remove hulls from germinated cottonseeds.

Acid-delinted cottonseeds with greater than 90-percent germination were soaked for 24 hours in water, drained, and then allowed to germinate for an additional 36 hours at 85% F. The germinated seeds were then blanched for 3 minutes and frozen. After removal from the freezer, the seeds were thawed and fed into the revolving rollers, which squeezed the meats from the hulls. The large rollers caused little damage to the immature cotyledons and left the empty seed hulls intact.

After the seeds had been processed through the rollers, they were placed in a large container filled with water. Most of the empty hulls floated because of the air trapped inside them, but the meats and a few hulls sank to the bottom of the container. The floating hulls were poured off and discarded. The settled material was placed on a large 4-mesh wire screen with an 80-mesh screen placed beneath it. By means of a strong jet of water, the flexible meats were washed through the top screen and deposited on the bottom screen. The rigid hulls were retained on the top screen. This method provided an approximate 96-percent separation of meats and hulls.

The meats, mixed with two parts of distilled water, were ground to a fine puree in a Waring Blendor. The 4 percent of hulls that remained in the mixture were removed by allowing the pieces to settle to the bottom of the blender and decanting the puree.

By this method, 35 to 40 pounds of seeds per hour could be dehulled as compared with less than 1 pound per hour by the hand-squeezing method.

Adult-Diet Press

Research by Vanderzant and Davich[7/] and Everett and Earle[8/] showed that the female weevil deposited more eggs on a curved surface than on a flat one. In their work, Vanderzant and Davich constructed plastic and aluminum molds for casting small diet cylinders. When the agar-base medium was poured by hand into the molds, it solidified as it cooled. The diet cylinders, removed from the molds, were later impaled on toothpicks and dipped into melted paraffin. This process, although satisfactory for maintaining small numbers of weevils, was too time consuming for the mass rearing of large numbers of weevils. Several other processes were therefore investigated.

At the State College laboratory, several attempts were made to reduce the time involved in preparing the diet cylinders. At first, the hot diet was poured into 4-foot lengths of glass tubing 8 mm. in diameter and allowed to cool. The cylinder thus formed was forced out of the tube and cut into 4-inch lengths. Each length was then coated with melted paraffin. Although this method took less time than the one with the individual molds, it was still too time consuming for mass rearing.

7/ See footnote 6.

8/ Everett, T. R., and Earle, N. W. Unpublished report.

Next, a stainless-steel press with a screw-driven plunger was constructed (fig. 3).

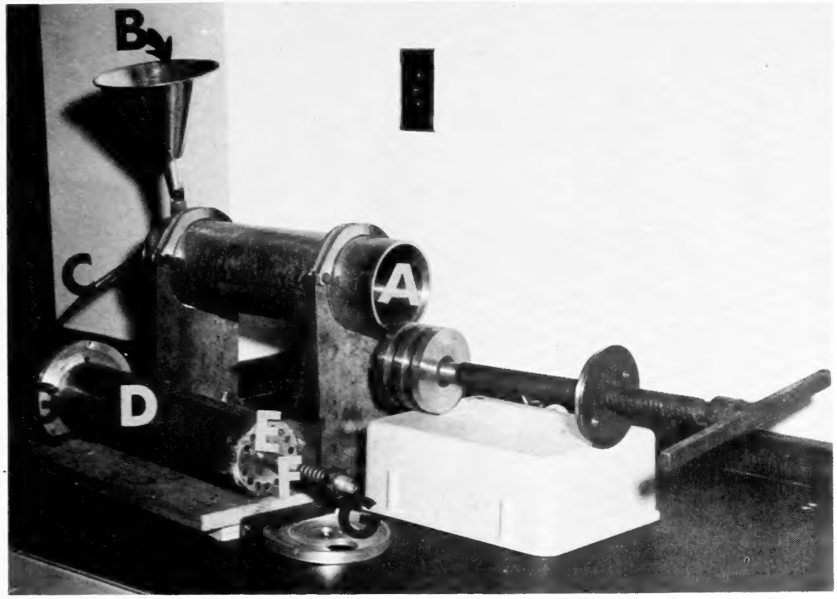

Figure 3.--View of adult diet press showing piston and cooling chamber with end plate removed: (A) Chamber; (B) funnel for adding diet; (C) copper tube; (D) large copper tube containing 15 smaller copper tubes; (E) center of large copper tube: (F) pipe used for axle and circulation of water inside chamber; (G) end plates.

The press, with a chamber (A) having a capacity of 2,500 ml., was built with a funnel (B) so that the melted diet could be poured directly into the chamber and then forced out through a $\frac{1}{4}$-inch pipe fitting (C) in the end plate (G). One end of a 25-foot length of $\frac{1}{4}$-inch copper tubing was fastened to the pipe fitting, coiled, and immersed in a tank of cold water. It was hoped that the hot medium could be forced through the tubing, cooled by the water, and be extruded as a continuous cylindrical rod of diet. The continuous moving of the medium as it cooled in the tube caused the partially solidified mass to crumple and emerge from the tube as a paste.

A revolving cooling device (fig. 3) was then constructed, for which a large copper tube (D) 3 inches in diameter and 18 inches long served as the chamber. Two end plates were fastened to the chamber and 15 smaller copper tubes one-fourth inch in diameter were each inserted through the end plates of the large copper tube so that they were all equidistant from its center (E). The resultant chamber resembled the barrels of a Gatling gun. A pipe one-half inch in diameter and 22 inches long (F) was run through the center of each end plate to act as an axle and also to permit cold water to be circulated inside the chamber and around the $\frac{1}{4}$-inch copper tubes.

A rubber hose connected one end of the pipe to a cold-water source, and another hose connected the other end of the pipe to a drain. The pipe had an obstruction near the center and holes drilled through the pipe wall on either side of the obstruction. Cold water would enter the chamber through one set of holes, circulate around the $\frac{1}{4}$-inch tubes, and then would leave the chamber through the other set of holes in the drain side of the pipe. The ends of the axle were mounted between two stationary end plates (G). The plates were pressed against the ends of the chamber by means of coil springs on the pipe axle. Single holes in each of the stationary end plates were situated so that one of the tubes in the revolving chamber could be aligned with these holes. A short length of $\frac{1}{4}$-inch copper tubing was used to connect the hole in one of the stationary end plates with the $\frac{1}{4}$-inch pipe fitting in the end of the press.

As the adult-diet medium was forced from the press, one of the tubes was filled in the cooling chamber. The chamber was then turned one-fifteenth of a revolution to present a new tube for filling. By the time all 15 tubes were filled, the material in the first tube had solidified and was forced out of the cylinder by another filling of hot medium. By this method, about 75 feet of $\frac{1}{4}$-inch diet cylinders per minute could be formed.

EGG-EXTRACTION APPARATUS

Gast[9/] developed a mechanical means of extracting boll weevil eggs from squares by using a Waring Blendor to grind up the squares. He then separated the eggs from the plant material by passing the material in salt solutions through screens. Subsequent work showed that more eggs could be obtained from small bolls than from squares. The blender method of grinding the small bolls was not satisfactory, since the grinding process caused considerable damage to the eggs and greatly reduced subsequent egg hatch.

Razor blades were first used for chopping the bolls into small pieces. The eggs could then be collected from the boll pieces without injury by washing the eggs from the chopped pieces through a series of screens. Because a large number of bolls had to be processed, the hand-chopping method required too much time. A machine for slicing the bolls was needed. Available commercial food-slicing equipment was unsatisfactory for this purpose, because the bolls could not be sliced thin enough. The equipment also tended to crush the bolls, since the blades were not as sharp as razor blades.

9/ Gast, R. T. Some shortcuts in laboratory rearing of the boll weevil. Jour. Econ. Ent. 54: 395-396. 1961.

A boll slicer (fig. 4) was constructed by mounting three razor blades (H) on a revolving aluminum disk (I).

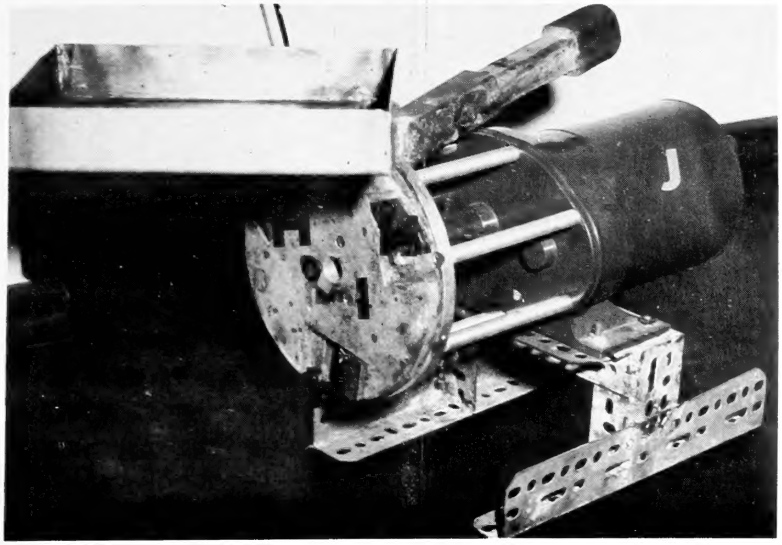

Figure 4.--Slicer used to cut cotton bolls for extraction of boll weevil eggs: (H) Razor blades; (I) revolving aluminum disk; (J) motor.

This disk was fastened to the shaft of a $\frac{1}{4}$-horsepower motor (J) geared to turn 120 revolutions per minute. Mounted to the motor was a stationary disk, in which a $1\frac{1}{2}$-inch hole had been cut to allow the bolls to be fed into the revolving razor blades. A hopper and chute were fastened to the stationary plate to facilitate feeding the bolls into the slicer.

Groups of bolls were sliced into different thicknesses to determine the most effective boll width from which to obtain the eggs. Slices 1 mm. thick furnished the most eggs during the washing process; however, because of the thinness of the slices, many eggs were cut. The numbers of eggs recovered increased as the thickness of the slices increased up to 2 mm., and then decreased as the slices became thicker because of the difficulty in washing the eggs from these thicker slices. When boll slices 2 mm. thick were used, approximately 65 percent of the eggs laid in the bolls were recovered by the slicing, washing, and screening process. In tests to determine the viability of the mechanically collected eggs, the average hatch was found to be 95 percent.

Newly hatched boll weevil larvae are cannibalistic and thus have to be separated for placement in the larval diet medium so that they will not contact each other. Since larvae are legless, they cannot cling to the agar-base medium in order to bite off pieces from its smooth surface. The larvae must be placed on a rough surface so that they can push the dorsal surface of their thoraxes against the medium and thus obtain sufficient leverage to force their mandibles into the medium. Workers have satisfied the requirements of the larvae by placing them in individual holes punched in the medium. Vanderzant and Davich 10/ and Earle et al. 11/ used small shell vials partially filled with larval medium. One egg was placed in each vial in a small hole punched in the medium. Brazzel et al. 12/ used petri dishes containing larval medium punched with 50 holes to receive the eggs. For both types of rearing equipment, the eggs were picked up individually on a sable-hair brush or dissecting needle and placed in the holes in the medium. An experienced technician could plant from 500 to 600 eggs per hour in either of these ways.

Because the planting of as many as 20,000 eggs a day entailed large labor costs, a less costly method was sought. The methods tried included mixing the eggs with granulated, moist diet medium; mixing them with dry medium to which water was later added; and suspending them in different solutions applied so as to flood the surface of the medium. None of these methods proved successful. A machine was then constructed to aid in planting the eggs.

The basic unit of the machine (fig. 5) consisted of a circular brass chamber (K), 3 inches in diameter and 1 inch in height, with a top and bottom plate. Evenly spaced holes were drilled in the bottom plate of the chamber. Into these holes seventy-eight 23-gauge, stainless-steel hypodermic needles (L) were inserted so that they extended one-half inch above and below this plate. The needles were then soldered in place. All needles were ground to exactly the same length and the ends flattened. Three adjustable legs (M) were fastened to the sides of the chamber so that when the legs were resting on a flat surface, the ends of all the needles were 0.8 mm. above the surface. Two $\frac{1}{4}$-inch copper tubes (N) were soldered to the top plate of the chamber, with one of the tubes extending inside the chamber within 1 mm. of the bottom plate. Rubber tubing, connected to the copper tube extending inside the chamber, was in turn connected to a vacuum source. The other copper tube was similarly connected to a compressed-air source.

Two microswitches (O), also mounted on the chamber, allowed the operator to turn them on and off with his fingertips while holding the chamber in his hand. One

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10/ See footnote 2.

11/ See footnote 4.

12/ Brazzel, J. R., Davich, T. B., and Raven, Klaus. Rearing the boll weevil on an artificial diet. Tex. Agr. Expt. Sta. Misc. Pub. 353, 4 pp. 1959.

microswitch actuated a solenoid valve in the vacuum line and the other a similar valve in the air line. The remainder of the unit consisted of a flat-bottomed plastic petri dish (P), 6 inches in diameter.

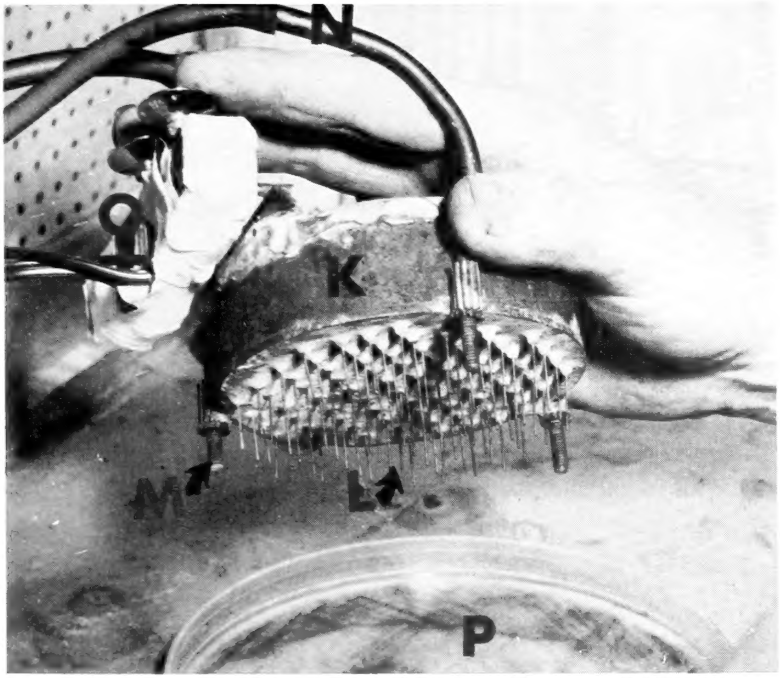

Figure 5.--Mechanical egg planter: (K) Circular brass chamber; (L) stainless-steel hypodermic needles; (M) one of three adjustable legs; (N) one of two copper tubes soldered to top plate of chamber; (O) two microswitches for activating solenoid valve in vacuum line and one in air line; (P) petri dish.

To pick up eggs with the planter, the operator first deposited the eggs in sterile water in the petri dish, placed the egg planter in the dish, and actuated the vacuum while moving the planter around the dish. As the ends of the needles contacted the eggs, the eggs were drawn to the needles and held. When each needle tip had picked up an egg, the operator removed the planter from the dish and held it over the larval medium. A short blast of compressed air then forced the eggs onto the surface of the medium.

Excess water drawn into the egg-planter chamber in the process of picking up the eggs was removed by the vacuum line. The only water deposited on the medium was the small amount retained in each of the needles.

Since newly hatched larvae cannot penetrate the smooth surface of the medium, it was necessary to thoroughly scratch the surface of the medium before the eggs were placed on it. This procedure was accomplished by using a small tool consisting of a plate with numerous sharp-pointed nails soldered to it. When this device was passed a few times over the surface of the medium, many hundred shallow fissures were formed in the medium. Thus, the young larvae could penetrate the medium.

With the egg-planting device, more than 1,000 eggs per minute could be planted in the diet medium as compared with 500 per hour by the hand method.

In a test comparing the effectiveness of machine planting with that of hand planting, 6,000 eggs were divided into two groups of 3,000 $\pm$ 20. One group was planted by hand and one by the planting device described above.

The data from this test were as follows:

	By hand	Mechanically
Dishes planted	38	41
Dishes that became contaminated	17	4
Time necessary for planting	$4\frac{1}{2}$ hours	5 minutes
Adult weevils that emerged	1,086	1,059
Average weight of adults	15.3 mg.	14.7 mg.

These data show that mechanical planting of eggs had little effect on weevil development.

In addition to the great saving in labor cost furnished by the egg-planting machine, the data show that a second important advantage was realized. The mechanical planter was responsible for a reduction in the number of petri dishes that become contaminated with molds and yeast. Most of this type of contamination is caused by airborne spores. The mechanically planted plates were open and exposed to such contamination for less than 2 seconds. In contrast, the hand-planted plates were open and exposed for as long as 5 minutes.

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